PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12Q 1/68, C12M 1/02, 1/38
B01L 7/00

A1

(11) International Publication Number: WO 91/07504

(43) International Publication Date: 30 May 1991 (30.05.91)

(21) International Application Number: PCT/AU90/00560

(22) International Filing Date: 20 November 1990 (20.11.90)

(30) Priority data:
PJ 7505 21 November 1989 (21.11.89) AU

(71) Applicant *(for all designated States except US)*: KINDCONI PTY. LTD. [AU/AU]; Unit 14, 2 Railway Parade, Lidcombe, NSW 2141 (AU).

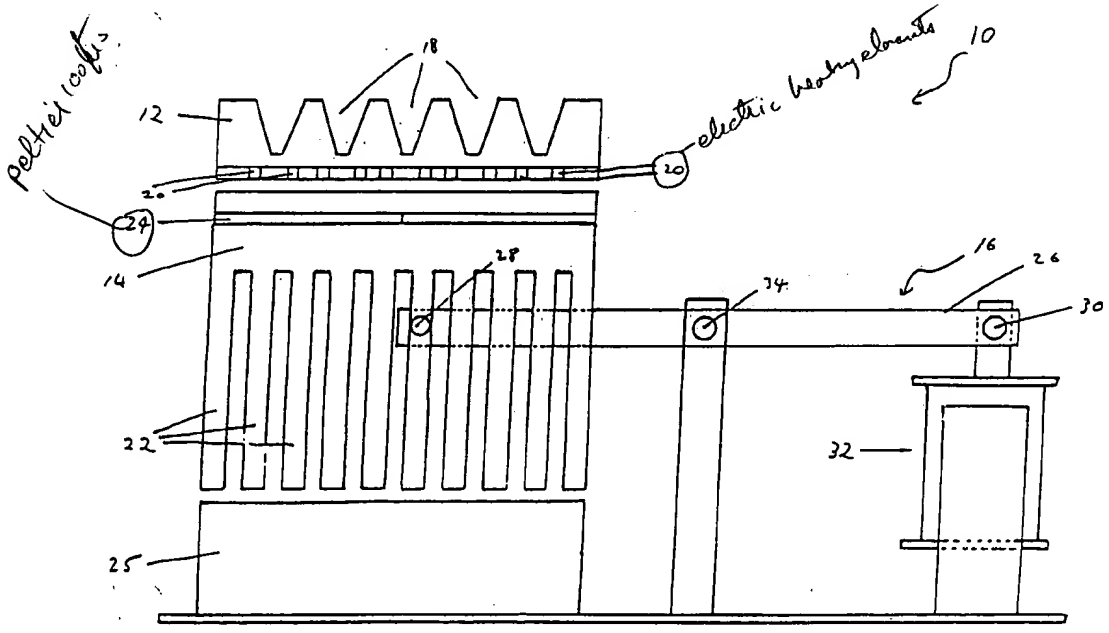
(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : CORBETT, John, Michael [AU/AU]; 39 Renwick Street, Drummoyne, NSW 2047 (AU).

(74) Agent: F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IMPROVED DNA POLYMERISATION DEVICE



(57) Abstract

The present invention provides a device for use in DNA polymerisation. The device comprises an element (12) adapted to receive tubes in which the DNA polymerisation reaction is to take place. The element (12) is provided with heating means (20) adapted to heat the element (12). The device also includes a heat sink (14) adapted to remove heat from the element (12) and movement means (16) enabling the movement of the element (12) and the heat sink (14) into and out of contact with each other.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	SD	Sudan
CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo			SN	Senegal
CH	Switzerland	KR	Republic of Korea	SU	Soviet Union
CI	Côte d'Ivoire	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		
ES	Spain	MG	Madagascar		

IMPROVED DNA POLYMERISATION DEVICE

Field of the Invention

The present invention relates to a device for use in DNA polymerisation.

Background of the Invention

In a number of applications, such as gene analysis and DNA fingerprinting, it is often necessary to multiply the amount of DNA present a sample. A DNA segment of up to approximately six thousand base pairs in length may be amplified exponentially starting from as little as a single gene copy by means of polymerised chain reaction.

In this technique a denatured DNA sample is incubated with two oligonucleotide primers that direct the DNA polymerase-dependent synthesis of complimentary strains. Multiple cycles of synthesis each afford an approximate doubling of the amount of target sequence. Each cycle is controlled by simply varying the temperature to permit denaturation of the DNA strands, annealing of the primers, and synthesis of new DNA strands. The use of a thermostable DNA polymerase obviates the necessity of adding new enzyme for each cycle, thus enabling fully automated DNA amplification. Twenty-five amplification cycles increase the amount of target sequence by approximately 10^6-fold. For the purposes of gene analysis the polymerase chain reaction technique offers the advantage of an increased signal intensity in subsequent assays. More detailed information regarding the polymerase chain reaction can be found in "PCR Protocols - A Guide to Methods and Applications" Eds. M.A. Innis, D.H. Gelfard, J.J. Sainskey, T.J. White, Academic Press. Inc. San Diego 1990" the disclosure of which is incorporated herein by reference.

Devices for use in DNA polymerisation typically consist of a heat conductive material provided with channels adapted to receive vessels in which the reaction

is to take place, typically Eppendorf tubes. The heat conductive material is then provided with heating/cooling means. One of the main difficulties encountered in the use of such devices has been the achievement of relatively rapid cooling of the DNA reaction mixture. The most common solution to this problem has been to use Peltier effect heat pumps to effect both the heating and the cooling of the reaction mixture. However, the continual cycling between heating and cooling typically leads to the Peltier effect heat pumps failing.

Summary of the Invention

The present inventor has developed a novel device which enables rapid heating and cooling of the DNA reaction mix whilst being relatively robust and economic to produce.

Accordingly, in a first aspect the present invention consists in a device for use in DNA polymerisation, the device comprising an element adapted to receive tubes in which the DNA polymerisation reaction is to take place and being provided with heating means adapted to heat the element, a heat sink adapted to remove heat from the element and movement means enabling the movement of the element and the heat sink into and out of contact with each other.

In a second aspect the present invention consists in a method of DNA amplification using polymerase chain reaction, the method being characterised in that a reaction mixture comprising DNA, suitable thermostable DNA polymerases and oligonucleotide primers in a vessel is placed in an element adapted to receive the vessel, the element being provided with heating means, heating the reaction mixture by actuation of the heating means, stopping the heating means, cooling the reaction mixture by bringing a heat sink into contact with the element, moving the heat sink away from the element and cyclically

repeating the heating and cooling steps.

In a preferred embodiment of the present invention the element remains stationary and the heat sink is moved into and out of contact with the element.

In a further preferred embodiment of the present invention the mass of the heat sink is substantially greater than the mass of the element. It is presently preferred that the mass of the heat sink is two to three times the mass of the element.

In yet a further preferred embodiment of the present invention the device is provided with means for cooling the heat sink. This cooling means may consist of a fan directed on the heat sink, Peltier effect coolers provided within the heat sink or other forms of cooling readily known in the art.

In yet another preferred embodiment of the present invention the movement of the element and/or the heat sink into and out of contact with each other is automated. Preferably, in operation, temperature sensing means is provided in the DNA reaction mixture. The temperature sensing means is preferably connected to control means which controls the movement of the element and/or heat sink into and out of contact with each other. It is preferred that this automation is computer controlled.

Detailed Description of the Invention

In order that the nature of the present invention may be more clearly understood, a preferred form thereof will now be described with reference to the accompanying drawing, in which is shown a schematic representation of a preferred embodiment of the device of the present invention.

The device 10 for use in DNA polymerisation comprises an element 12, a heat sink 14 and movement means 16. The element 12 and heat sink 14 are made of material which is a good thermal conductor, such as metal.

The element 12 is provided with wells 18 adapted to receive tubes in which the DNA polymerisation reaction is to take place. The element 12 is also provided with heat electric heating elements shown generally as 20.

The heat sink 14 is provided with cooling fins 22 to enable a greater dissipation of heat from the heat sink. The cooling fins 22 are positioned above a fan 25 arranged such as to blow air onto the cooling fins 22 of the heat sink 14.

The heat sink 14 is also provided with Peltier effect coolers shown generally as 24.

The movement means 16 enables the movement of the heat sink 14 into and out of contact with the element 12. The movement means 16 consists of arm 26 connected at end 28 to heat sink 14 and at end 30 to actuator 32. The arm 26 is pivoted about point 34. Actuation of actuator 32 will result in the heat sink 14 being brought into contact with the element 12.

In order that the nature of the operation of the device of the present invention may be more clearly understood the operation of the device shown in the accompanying drawing will now be described.

A reaction mixture of DNA, thermostable DNA polymerase and oligonucleotide primers in tubes are placed in wells 18 of element 12. The electric heating elements 20 are then actuated to raise the temperature of the element 12 and consequently the temperature of the reaction mix in the tubes in wells 18. The temperature is then maintained at a predetermined level, typically 93°C for a predetermined period of time, typically one and a half minutes. At the end of the heating period the electric heater elements 20 are turned off and actuator 32 actuated resulting in bringing heat sink 14 into contact with element 12. As heat sink 14 is at a substantially lower temperature than element 12 heat is lost from

element 12 to heat sink 14. The reaction mixture is cooled to a predetermined temperature, typically 60°C and held at this temperature for a predetermined period of time, typically one and a half minutes.

Typically, given the substantially greater mass of the heat sink 14 in comparison to element 12, the adsorption of heat from element 12 by heat sink 14 results in little raising of the temperature of heat sink 14. In addition, heat is rapidly lost from the heat sink 14 due to the action of fan 25 directing air on cooling fins 22. In addition, the temperature of the heat sink 14 may also be lowered by Peltier coolers 24.

At the end of the cooling step the actuator 32 is once again actuated and heat sink 14 is moved out of contact with element 12. At this time the electric heater elements are again actuated and the reaction mixture heated once again.

These steps are then cyclically repeated until the desired number of amplification steps are achieved.

In use the device of the present invention has been shown to be capable of achieving the drop in temperature from 93°C to 60° in approximately thirty seconds without the use of the Peltier effect coolers. As will be appreciated by persons skilled in the art this is a substantial improvement over prior art devices.

As would be appreciated by persons skilled in the art the cyclic nature of the operation of the device of the present invention lends itself to computer automation. In such a situation the temperature of the element 12 and the DNA reaction mixture is monitored and the electric heating elements 20 cyclically actuated and the heat sink 14 cyclically brought into contact with the element 12 by means of movement means 16 to achieve cooling of the element 12 and consequently the reaction mixture.

As would be appreciated by persons skilled in the art

such DNA amplification procedures are typically run over an extended period of time to provide the required number of amplification cycles. If desired at the end of the required number of cycles, the computer can be programmed such that the heat sink 14 is brought into contact with the element 12 and the Peltier coolers 26 actuated to maintain the DNA reaction mixture at a predetermined temperature such as 4°C. Any risk of substantial denaturation of the amplified DNA would therefore be reduced.

As will be appreciated by persons skilled in the art the device of the present invention provides a relatively robust and simple alternative to devices of the prior art whilst providing a rapid means of cooling the DNA reaction mixture.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:-

1. A device for use in DNA polymerisation, the device comprising an element adapted to receive tubes in which the DNA polymerisation reaction is to take place and being provided with heating means adapted to heat the element, a heat sink adapted to remove heat from the element and movement means enabling the movement of the element and the heat sink into and out of contact with each other.

2. A device as claimed in claim 1, in which the element remains stationary and the heat sink is moved into and out of contact with the element.

3. A device as claimed in claim 1 or 2, in which the mass of the heat sink is substantially greater than the mass of the element.

4. A device as claimed in claim 3, in which the mass of the heat sink is two to three times the mass of the element.

5. A device as claimed in any one of claims 1 to 4, in which the heat sink is provided with cooling means selected from the group consisting of a fan directed on the heat sink, Peltier effect coolers provided within the heat sink or a combination thereof.

6. A method of DNA amplification using polymerase chain reaction, the method being characterized in that a reaction mixture comprising DNA, suitable thermostable DNA polymerases and oligo nucleotide primers in a vessel is placed in an element adapted to receive the vessel, the element being provided with heating means, heating the reaction mixture by actuation of the heating means, stopping the heating means, cooling the reaction mixture by bringing a heat sink into contact with the element, moving the heat sink away from the element and cyclically repeating the heating and cooling steps.

7. A method as claimed in claim 6, in which the element remains stationary and the heating sink is moved into and

out of contact with the element.

8. A method as claimed in claim 6 or claim 7, in which the mass of the heat sink is substantially greater than the mass of the element.

9. A method as claimed in claim 8, in which the mass of the heat sink is two to three times the mass of the element.

10. A method as claimed in any one of claims 6 to 9, in which the heat sink is provided with cooling means selected from the group consisting of a fan directed on the heat sink, Peltier effect coolers provided within the heat sink and combinations thereof.

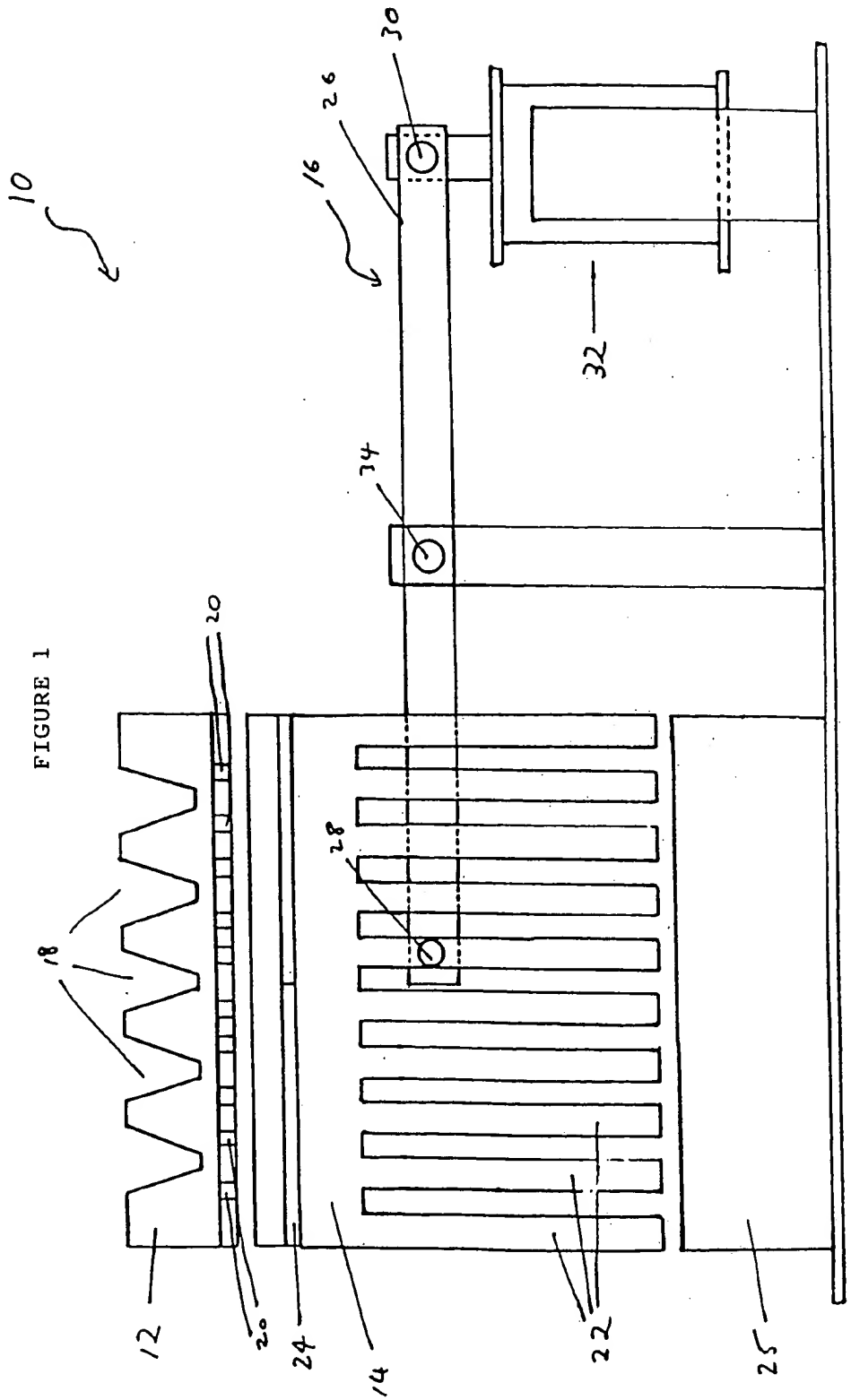


FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 90/00560

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C12Q 1/68, C12M 1/02, C12M 1/38, B01L 7/00

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC[5]	C12Q 1/68, C12M 1/02, C12M 1/38, C12M 1/40, C12M 1/36, B01L 9/00, 9/06, 7/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

WPAT DERWENT DATABASE KEYWORD SEARCH: MULLIS, PERKIN ELMER CETUS, CETUS CORP, POLYMER1:

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X	AU,A, 69180/87 (CETUS CORPORATION) 27 August 1987 (27.08.87) See whole document, the figures in particular.	(1-10)
Y	Bio/Technology, Volume 6, No.1, January 1988, page 82, Top left-hand column illustrates above citation.	(1-10)
A	Bio/Technology, Volume 6, No.9, September 1988, page 1104, Top left-hand column illustrates a Hybaid thermal cycler.	(1-10)
X	US,A, 4865986 (COY et al) 12 September 1989 (12.09.89) See whole document, the abstract in particular.	(1-10)
P,X	WO,A, 89/12502 (LEP SCIENTIFIC LIMITED) 28 December 1989 (28.12.89) See whole document, the claims in particular. (continued)	(1-10)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
5 March 1991 (05.03.91)	19 March 1991
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	M. KEESE

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	DE,A, 3808942 (BIO-MED GMBH GESELLSCHAFT FUR BIOTECHNOLOGIE UND MEDIZINTECHNIK MBH) 28 September 1989 (28.09.89) See whole document, the abstract in particular.	(1-10)
P,X	DE,A, 3839162 (NOTGHI, A.) 23 May 1990 (23.05.90) See whole document	(1-10)
Y	EP,A, 342155 (AGROGEN-STIFTUNG) 15 November 1989 (15.11.89) See whole document, the figures in particular.	(1-10)
Y	Nature, Volume 331, 4 February 1988 (04.02.88), pages 461-462. H.A. Erlich et al, "Specific DNA amplification".	(1-10)

V. [ ] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.[ ] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2.[ ] Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.[ ] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1.[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL APPLICATION NO. PCT/AU 90/00560

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
DE 3808942			
DE 3839162			
US 4865986	EP 363143	JP 2176910	
WO 8912502	AU 38382/89	GB 8814962	
EP 342155	CH 676332		
AU 69180/87	DK 978/87 JP 62240862	EP 236069 NZ 219388	IL 81663